UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 27 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Response to the Bromacil Reregistration Standard: Residue Chemistry Studies (MRID #'s 42967301, 42967501 and 42967401, CBRS #'s 12786, 12787 and 12802, Barcodes: D196552, D196549 and D196553).

FROM: R. B. Perfetti, Ph.D., Chemist
Reregistration Section 2
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

THRU: William J. Hazel, Ph.D., Section Head
Reregistration Section 2
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

TO: Esther Saito, Chief
Reregistration Branch
Special Review & Reregistration Division (7508W)

Attached are three reviews of residue chemistry studies submitted in response to the bromacil Reregistration Standard. These reviews were completed by Dynamac Corporation under supervision of CBRS, HED. They have undergone secondary review in the branch and have been revised to reflect Agency policies.

1. The qualitative nature of bromacil residues in/on oranges is adequately understood. Total radioactive residues (expressed as bromacil equivalents) are readily taken up by fully grown orange trees and translocated into leaves and fruits following soil application of [2-^{14}C]bromacil to established orange trees at 1x the maximum registered rate for the CA test site. The total ^{14}C-residues peaked 34 days posttreatment at 0.044 ppm in/on fruits.

2. Virtually all of the total ^{14}C-residues in/on orange fruits were adequately characterized. The major metabolites identified were the malonyl glucose conjugate of Metabolite A (58-92% TRR) and the glucose conjugate of Metabolite A (1-21% TRR). Several minor peaks were poorly resolved; however,

these peaks were present at <0.05-0.016 ppm. Neither parent bromacil nor Metabolite A in its free form were detected in orange fruits.

3. Based on the low levels of the two conjugates of metabolite A observed in this study, and since corresponding animal conjugates of this metabolite were observed in rats and goats, it is concluded that these compounds need not be included in the tolerance expression (Personal discussions, A. Protzel (TOX 2), L. Taylor (TOX 2) and K. Baetcke (TOX 1). Therefore the residue to be regulated in citrus is bromacil.

4. Since no residues of bromacil were observed in the **citrus** metabolism study, there is no reason that representative samples from this study need to be analyzed by the tolerance enforcement method(s) to confirm that the method(s) adequately recover the parent.

5. The modified GLC/ECD method has a more sensitive limit of detection (LOD = 0.01 ppm) than any of the enforcement methods published in PAM Vol. II and is adequate for data collection of residues of bromacil *per se* in/on oranges and pineapple. Acceptable method recoveries were obtained in oranges (83-110%) and pineapple (80-110%) following fortifications with bromacil at 0.01-0.12 ppm.

6. Since bromacil is the residue to be regulated on citrus, CBRS recommends that this modified GLC/ECD method undergo an independent laboratory method validation in accordance with PR Notice 88-5. If the required pineapple metabolism study in progress indicates that additional pineapple metabolites need to be included in the tolerance expression, then adequate residue analytical methods capable of determining these residues must be developed for pineapples.

7. The submitted storage stability data indicate that fortified residues of bromacil *per se* are stable under frozen storage condition (-15 C) for up to 18 months in/on citrus fruits and pineapple, the only crops on which bromacil is registered for use.

8. A magnitude of the residue study in citrus fruits is presently not required. The registrant did not provide information regarding the duration of storage of citrus samples that were used for tolerance assessment. In consideration of the facts that no bromacil was observed in the citrus metabolism study and that residue samples used for tolerance assessment mostly bore nondetectable (<0.05 ppm) residues of bromacil *per se*, it can be concluded that the citrus fruit field trials are validated by acceptable storage stability data.

9. A pineapple field residue study is ongoing. CBRS recommends that harvested samples from the pineapple field trials be analyzed within 18 months or storage stability data reflecting longer storage intervals will be required.

10. The qualitative nature of the residue in **citrus** is adequately understood. If the pineapple metabolism study in progress indicates that additional metabolites need to be included in the tolerance expression, then additional storage stability data on these residues will be required.

If you need additional input please advise.

Attachments: Bromacil Residue Chemistry Reviews

cc (With Attachments): RBP, Bromacil Reregistration Standard File, Bromacil Subject File, RF, Circ. and Dynamac.

DYNAMAC
CORPORATION
Environmental Services

Final Report

BROMACIL
Shaughnessy No. 012301; Case No. 0041 (CBRS No. 12802; DP Barcode D196549)

TASK 4
Registrant's Response to Residue Chemistry Data Requirements

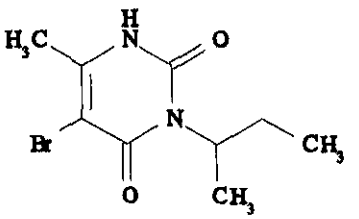
January 26, 1994

Contract No. 68-D2-0053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
2275 Research Boulevard
Rockville, MD 20850-3268

BROMACIL



Shaughnessy No. 012301; Case 0041

(CBRS No. 12802; DP Barcode D196549)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

E. I. du Pont de Nemours and Company has submitted data depicting the metabolism of [^{14}C]bromacil in oranges (1993; MRID 42967501) in response to the Second Round Review (SRR) of the Bromacil Reregistration Standard dated 8/15/89. CBRS had previously commented on the specifics and details of the protocol (J. Smith, DP Barcode D177412 dated 6/3/92; S. Knizner, DP Barcodes D181056 dated 8/6/92 and D183799 dated 2/9/93) prior to the initiation of this orange metabolism study. The submitted orange metabolism data are the subject of this evaluation and the Conclusions and Recommendations stated in this document pertain only to this topic. All other residue chemistry data requirements required in the Bromacil Reregistration Standard SRR are not addressed herein.

A pineapple metabolism study is currently in progress and will be available for review by 9/94. The qualitative nature of the residue in ruminants is adequately understood. This is a category 3 situation with respect to meat and milk, and therefore no tolerances are needed for these commodities. A poultry metabolism study is presently not required since bromacil is not registered for use on crops that are used as poultry feed.

Tolerances for residues of bromacil in/on plant commodities are expressed in terms of bromacil *per se* [40 CFR §180.210]. No tolerances in animal commodities have been established. Adequate methods are available for tolerance enforcement and data collection. A GLC method with microcoulometric detection is available for tolerance enforcement and is listed in Pesticide Analytical Method (PAM) Vol. II as Method I. Additional methods deemed adequate for purposes of tolerance enforcement include a GLC method with electron-capture detection, published in PAM Vol. II as Method B, and an improved GLC method using a thermionic nitrogen/phosphorus detector. These methods have not undergone validation by the Agency; therefore, they may be considered only as confirmatory methods for determining residues of bromacil *per se*.

CONCLUSIONS AND RECOMMENDATIONS

1. The qualitative nature of bromacil residues in/on oranges is adequately understood. Total radioactive residues (expressed as bromacil equivalents) are readily taken up by fully grown orange trees and translocated into leaves and fruits following soil application of [2-^{14}C]bromacil to established orange trees at 1x the maximum registered rate for the CA test site. The total ^{14}C-residues peaked 34 days posttreatment at 0.044 ppm in/on fruits.

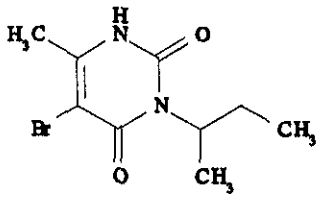
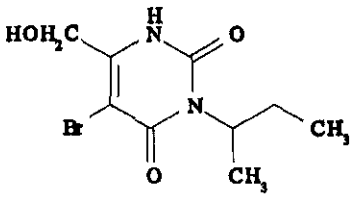
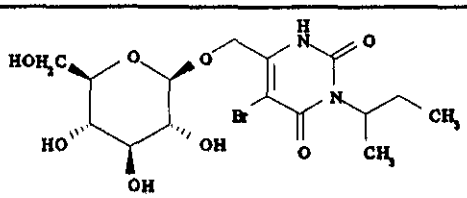
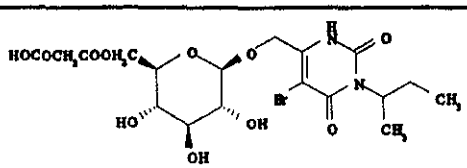
2. Virtually all of the total ^{14}C-residues in/on orange fruits were adequately characterized. The major metabolites identified were the malonyl glucose conjugate of Metabolite A (58-92% TRR) and the glucose conjugate of Metabolite A (1-21% TRR). Several minor peaks were poorly resolved; however, these peaks were present at $<$0.05-0.016 ppm. Neither parent bromacil nor Metabolite A in its free form were detected in orange fruits.

3. Based on the low levels of the two conjugates of metabolite A observed in this study, and since corresponding animal conjugates of this metabolite were observed in rats and goats, it is concluded that these compounds need not be included in the tolerance expression (Personal discussions, A. Protzel (TOX 2), L. Taylor (TOX 2) and K. Baetcke (TOX 1). Therefore the residue to be regulated in citrus is bromacil.

4. Since no residues of bromacil were observed in the **citrus** metabolism study, there is no reason that representative samples from this study need to be analyzed by the tolerance enforcement method(s) to confirm that the method(s) adequately recover the the parent.

The molecular structures of bromacil and its metabolites characterized and identified in/on orange fruits and leaves are presented in Table 1.

Table 1. Bromacil and its metabolites in oranges (MRID 42967501).

Common Name Chemical Name	Structure	Substrate
Bromacil 5-bromo-3-*sec*-butyl-6-methyluracil		Orange leaves
Metabolite A 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil		Orange leaves
Glucose conjugate of Metabolite A		Orange leaves and fruits
Malonyl glucose conjugate of Metabolite A		Orange leaves and fruits

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Plants

Directions for use

The following bromacil formulations (including MAI formulations) are registered for band or broadcast ground treatment beneath and/or between established bearing citrus trees at 1.6-6.4 lb ai/A/application or 2.4-3.2 lb ai/A/application by split treatments: (i) the 4% G (Reg. Nos. 352-410 and 352-412), (ii) the 40%, 53%, and 80% WP (Reg. Nos. 352-352, 352-351 and 352-287, respectively), and (iii) the 40%, 53%, and 80% DF (Reg. Nos. 352-505, 352-440, and 352-546, respectively) formulations. The application rates are dependent on soil types; higher rates are recommended for silt loam or clay loam soils and lower rates are recommended for sand or loamy sand soils. Spray applications may be made in a minimum of 40 gal of water/A. In addition, the 53% WP (MAI; EPA Reg. No. 352-351) formulation may be used in FL as a band or broadcast treatment at 4.2 lb ai/A/application. A maximum of 6.4 lb ai/A/season may be applied for band or broadcast treatment(s) for all formulations except the 53% WP formulation which allows up to 8.5 lb ai/A/season when applied in FL. No PHI has been established.

In-life phase

Du Pont submitted data (1993; MRID 42967501) depicting the metabolism of [^{14}C]bromacil in oranges. The in-life portion of the study was conducted by du Pont (Madera, CA). [2-^{14}C]Bromacil (radiochemical purity >97%, specific activity 31.3 μCi/mg) was diluted with unlabeled technical bromacil (unspecified purity) to yield a test substance with a specific activity of 2.39 μCi/mg. To prepare the treatment solution, the test substance was then formulated with the inert ingredients of the 80% WP formulation (EPA Reg. No. 352-287).

The test plot was of sandy loam soil type and consisted of an 8-foot diameter area with an established orange tree (8 years old and bearing fruits ready to be harvested within 1-4 months) in the middle. Prior to the application of the test substance, the test area was presoaked with 30 gal of water. The test substance was sprayed on the soil in the treatment plot under the orange tree at a rate of 4.8 lb ai/A. The applied rate is ca. 1x the maximum registered single/seasonal rates in CA for this soil type; however, the rate is 0.75x the maximum registered single/seasonal rates when applied in citrus-growing regions other than CA. [In the earlier review of the study protocol (S. Knizner, DP Barcode D183799, 2/9/93), CBRS acknowledged that an application rate greater than 4.8 lb ai/A has not been proposed by the registrant because of the limitations on the amount of radioactive material that can be applied to the "open" environment at the farm site in Madera, CA.] Immediately after treatment, the test area was sprinkle-irrigated with an additional 10 gal of water to soak the test material evenly in the soil. A similar size test plot containing a bearing orange tree served as control.

Oranges (2 inches in diameter to full size) and leaves were harvested at 0-, 7-, 19-, 34-, 61-, and 117-day posttreatment intervals (PTI). The fruits were full size at 34-day PTI. After harvest, all collected samples were frozen until shipped to Du Pont (Wilmington, DE), where the samples were stored frozen (-20 C) for a maximum of 40 days prior to analysis.

Total radioactive residues (TRR)

Subsamples of orange fruits and leaves from each sampling period were macerated in a blender with liquid nitrogen. Triplicate aliquots of homogenized fruits and leaves were then analyzed by combustion followed by liquid scintillation spectrometry (LSS) to determine total radioactive residues, which are presented in Table 2. The limit of detection for combustion/LSS was 0.007 ppm for orange fruits and 0.01 ppm for leaves.

Table 2. Total radioactive residues found in/on orange fruits and leaves harvested from an established orange tree treated with soil application of [^{14}C]bromacil at 4.8 lb ai/A.

Posttreatment Interval (Days)	[^{14}C]Bromacil Equivalents (ppm) * Based on Fresh-Weight Basis	
	Fruits	Leaves
0	0.007	0.01
7	0.015	0.45
19	0.042	2.24
34	0.044	5.77
61	0.038	2.55
117	0.039	2.94

* Average of triplicate analyses.

A subsample of 117-day PTI fruit (TRR = 0.039 ppm) was also fractionated into peel, pulp, and juice to determine residue distribution in these fractions; the TRR were determined by combustion/LSS. The distribution of radioactive residues reportedly were: peel (76.1% TRR, 0.030 ppm), pulp (18.4% TRR, 0.007 ppm), and juice (5.5% TRR, 0.002 ppm).

Extraction of ^{14}C-residues

The registrant provided descriptions of procedures and steps used for extraction of residues from orange fruits and leaves. During the extraction and fractionation, aliquots of extracts and non-extractable residues were analyzed by LSS and combustion/LSS, respectively. The 0-day PTI fruit samples were not extracted due to low (0.007 ppm) TRR values. Aliquots of homogenized fruits from the remaining sampling intervals and of leaves were separately extracted (3x) with acetonitrile:water (ACN:water; 90:10; v:v). The ACN:water extracts were combined and partitioned (3x) with hexane. The hexane washes were discarded and the

ACN:water fractions were concentrated, filtered, and reserved for HPLC analysis. The remaining non-extractable residues were air-dried and analyzed by combustion/LSS.

Characterization and identification of ^{14}C-residues in orange fruits

The ACN:water extracts of fruits were analyzed by HPLC using either a PRP-1 or an ODS C18 column with a PRP-1 or RP-18 guard column and a UV (277 nm or 280 nm) and/or Ramona-LS radioactivity detector. The solvent systems consisted of linear gradients of water:ACN from 90:10 to 85:15 over a period of 40 minutes (System I) or isocratic water:ACN (79:21; System II). The limit of detection for this analysis presumably (not reported) was 0.005 ppm.

Radioactive residues were identified by comparing the retention times with those of the following non-labeled reference standards: bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), Metabolite A (5-bromo-3-*sec*-butyl-6-hydroxymethyluracil), Metabolite C (5-bromo-3-(*a*-hydroxymethylpropyl)-6-methyluracil), Metabolite D (5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil, Metabolite F (3-*sec*-butyl-6-methyluracil, Metabolite G (5-bromo-6-methyluracil), and glucose conjugate of Metabolite A.

HPLC analysis of the fruit ACN:water extracts resolved two major peaks. A peak with R_t = 17 min was initially identified as the malonyl glucose conjugate of Metabolite A (0.008-0.062 ppm; 58-92% TRR). A peak with R_t = 22 min was initially identified as the glucose conjugate of Metabolite A (<0.005-0.012 ppm; 1-21% TRR). Several minor peaks (R_t = 11-15 min) were poorly resolved; however, these peaks were present at <0.05-0.016 ppm. The parent bromacil was not detected in citrus fruits.

The distribution of [^{14}C]bromacil residues found in/on orange fruits is presented in Table 3. Although the registrant provided quantitative characterization data for orange leaves, these data are not presented herein. However, these data are qualitatively discussed in the ensuing paragraph. A summary of the metabolites identified in the fruits is presented in Table 4. It is noted that the TRR from the fractionation procedure is slightly higher than the TRR obtained by combustion/LSS. The registrant explained that the orange fruit may not have been homogenous when sampled for combustion/LSS.

Isolation and characterization of major ^{14}C-residues in orange leaves

The registrant also conducted isolation and characterization of ^{14}C-residues in orange leaves to confirm the metabolites initially identified in the fruits. The low TRR values in fruits precluded the registrant from conducting confirmatory procedures using fruit samples.

HPLC analysis of the initial leaf ACN:water extracts resolved: (i) malonyl glucose conjugate of Metabolite A (R_t = 17 min at 0.14-3.58 ppm; 46-100% TRR); (ii) glucose conjugate of Metabolite A (R_t = 22 min at ND-0.54 ppm; up to 23% TRR); (iii) several minor peaks (R_t = 10-13 min at ND-0.64 ppm; up to 18% TRR); (iv) bromacil (only on Day-7 samples at

0.068 ppm; 23% TRR); and (v) Metabolite A (only on Day-34 samples at 0.93 ppm; 16% TRR). Except for the presence of bromacil and Metabolite A, the metabolites identified in the leaves are consistent with those identified in the fruits.

Bromacil and Metabolite A were confirmed by co-chromatography with reference standards using one-dimensional TLC on silica gel plates with a methylene chloride:ethyl acetate (1:3; v:v) solvent system. In addition to TLC, these residues were confirmed by comparison with reference standards using HPLC (System II) and LC/MS. Sample chromatograms and mass spectra were included in the submission.

Additional leaves were extracted as previously described to collect and isolate fractions corresponding to the 17- and 22-min HPLC peaks. In addition, fractions from the partially purified 22-min peak were subjected to enzyme (β-glucuronidase) and acid (1 N HCl) hydrolysis and then analyzed by HPLC (System I). Both hydrolyses procedures yielded Metabolite A, confirming that the peak with R_t = 22 min is a glucose conjugate of Metabolite A. However, LC/MS analysis of the 22-min peak indicated that it is a mixture of glucose conjugate of Metabolite A and its acetate ester. Further analysis of the 22-min peak from day-117 leaves by HPLC (System II) also indicated the presence of two compounds, one with R_t = 5.55 min corresponding to the glucose conjugate of Metabolite A and another with R_t = 6.49 min which is most likely to be the acetate ester of glucose conjugate of Metabolite A. The registrant explained that the presence of acetate glucose conjugate of Metabolite A is either an artifact of the isolation process or a degradation product of the malonyl glucose conjugate of Metabolite A (R_t = 17 min).

Finally, the 22-min peak from day-117 orange fruit extract was analyzed by HPLC (System 1) and spiked with a purified 22-min peak fraction from leaves. The results showed an enhancement of the 22-min peak, indicating that the 22-min peak in orange fruits and leaves are identical.

Attempts to use the purified 17-min peak from leaf samples to confirm the identity of the 17-min peak from fruits were unsuccessful because the isolated ^{14}C-residue was found to be relatively unstable; see "Storage stability" section. The registrant, however, stated that in a previous orange fruit metabolism study conducted under greenhouse conditions, a similar 17-min HPLC fraction had been isolated and identified by thermospray LC/MS as the malonyl glucose conjugate of Metabolite A.

Table 3. Distribution of total radioactive residues in/on orange fruits harvested from an established orange tree treated with soil application of [^{14}C]bromacil at 4.8 lb ai/A.

Fraction	%TRR	ppm	Characterization/Identification [a]
Oranges, 7-day PTI (0.015 ppm)			
ACN:water	96.8	0.015	Identified malonyl glucose conjugate of Metabolite A (57.6 %TRR, 0.008 ppm) and glucose conjugate of Metabolite A (20.5 %TRR, <0.005 ppm); resolved a polar fraction (3.3 %TRR, <0.005 ppm), and others (18.6 %TRR, <0.005 ppm)[b].
Non-extractable	3.2	0.0005	N/A = Not analyzed
Oranges, 19-day PTI (0.042 ppm)			
ACN:water	98.0	0.051	Identified malonyl glucose conjugate of Metabolite A (72.2 %TRR, 0.036 ppm) and glucose conjugate of Metabolite A (20.5 %TRR, 0.010 ppm); resolved a polar fraction (5.8 %TRR, <0.005 ppm), and others (1.4 %TRR, <0.005 ppm).
Non-extractable	2.0	0.001	N/A
Oranges, 34-day PTI (0.044 ppm)			
ACN:water	97.7	0.082	Identified malonyl glucose conjugate of Metabolite A (67.2 %TRR, 0.055 ppm) and glucose conjugate of Metabolite A (14.7 %TRR, 0.012 ppm); resolved a polar fraction (13.1 %TRR, 0.011 ppm), and others (5.0 %TRR, <0.005 ppm).
Non-extractable	2.3	0.002	N/A
Oranges, 61-day PTI (0.038 ppm)			
ACN:water	95.2	0.068	Identified malonyl glucose conjugate of Metabolite A (92.1 %TRR, 0.062 ppm) and glucose conjugate of Metabolite A (0.8 %TRR, <0.005 ppm); resolved a polar fraction (2.1 %TRR, <0.005 ppm), and others (5.0 %TRR, <0.005 ppm).
Non-extractable	4.8	0.003	N/A
Oranges, 117-day PTI (0.039 ppm)			
ACN:water	94.0	0.085	Identified malonyl glucose conjugate of Metabolite A (63.8 %TRR, 0.054 ppm) and glucose conjugate of Metabolite A (13.9 %TRR, 0.012 ppm); resolved a polar fraction (19.4 %TRR, 0.016 ppm), and others (2.9 %TRR, <0.005 ppm).
Non-extractable	6.0	0.005	N/A

[a] The HPLC (System I) retention times of metabolites are: 17-18 min (malonyl glucose conjugate of Metabolite A, 22 min (glucose conjugate of Metabolite A), and 10-15 min (polar fraction). Metabolites in fruits were confirmed by HPLC co-chromatography of metabolites identified from leaves.

[b] HPLC (System II) and LC/MS analyses indicate that glucose conjugate of Metabolite A was found to occur in combination with its acetate ester.

Table 4. Summary of characterization and identification of radioactive residues in/on orange fruits harvested from an established orange tree treated with soil application of [^{14}C]bromacil at 4.8 lb ai/A.

Metabolite	7-day PTI		19-day PTI		34-day PTI		61-day PTI		117-day PTI	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified										
Glucose conjugate of Metabolite A	20.5	<0.005	20.5	0.010	14.7	0.012	0.8	<0.005	13.9	0.012
Malonyl glucose conjugate of Metabolite A	57.6	0.008	72.2	0.036	67.2	0.055	92.1	0.062	63.8	0.054
Total identified	**78.1**	**<0.013**	**92.7**	**0.046**	**81.9**	**0.067**	**92.9**	**<0.067**	**77.7**	**0.066**
Characterized										
Polar fraction	3.3	<0.005	5.8	<0.005	13.1	0.011	2.1	<0.005	19.4	0.016
Other unknowns	18.6	<0.005	1.4	<0.005	5.0	<0.005	5.0	<0.005	2.9	<0.005
Total identified/ characterized	**100.0**	**<0.023**	**99.9**	**<0.056**	**100.0**	**<0.083**	**100.0**	**<0.077**	**100.0**	**<0.087**
Non-extractable	3.2	0.0005	2.0	0.001	2.3	0.002	4.8	0.003	6.0	0.005

Proposed metabolic pathway

The registrant proposes a metabolic pathway for bromacil in orange fruits. Bromacil undergoes hydroxylation at the 6-methyl group to produce Metabolite A, which is readily conjugated with glucose and is converted to malonyl ester of glucose conjugate of Metabolite A.

Storage stability

Samples from this metabolism study were stored frozen (-20 C) until analysis. The dates of sample collection and analysis were provided. It is our calculation that residue extraction and characterization were completed within 40 days of harvest.

The storage stability of frozen 117-day PTI leaf samples were investigated. On initial analysis, the leaf contained only the 17-min peak metabolite (malonyl glucose conjugate of Metabolite A). Following 5 months of frozen storage, the leaf samples were re-extracted and analyzed by HPLC as previously described. The analysis indicate that the initial residue was converted to Metabolite A and its glucose conjugate.

Study summary

The qualitative nature of bromacil residues in/on oranges is adequately understood. Total radioactive residues (expressed as bromacil equivalents) are readily taken up by fully grown orange trees and translocated into leaves and fruits following soil application of [2-^{14}C]bromacil to established orange trees at 1x the maximum registered rate for the CA test site. The total ^{14}C-residues peaked 34-day posttreatment at 0.044 ppm in/on fruits.

Virtually all of the total ^{14}C-residues in/on orange fruits were adequately characterized. The major metabolites identified were the malonyl glucose conjugate of Metabolite A (58-92% TRR) and the glucose conjugate of Metabolite A (1-21% TRR). Several minor peaks were poorly resolved; however, these peaks were present at $<$0.05-0.016 ppm. Neither parent bromacil nor Metabolite A in its free form were detected in orange fruits.

Based on the low levels of the two conjugates of metabolite A observed in this study, and since corresponding animal conjugates of this metabolite were observed in rats and goats, it is concluded that these compounds need not be included in the tolerance expression (Personal discussions, A. Protzel (TOX 2), L. Taylor (TOX 2) and K. Baetcke (TOX 1). Therefore the residue to be regulated in citrus is bromacil.

Since no residues of bromacil were observed in the **citrus** metabolism study, there is no reason that representative samples from this study need to be analyzed by the tolerance enforcement method(s) to confirm that the method(s) adequately recover the the parent.

EPA MEMORANDA CITED IN THIS REVIEW

CBRS No.: 9778; DP Barcode D177412
Subject: Bromacil. Protocol for Nature of the Residue in Plants - Citrus Fruit.
From: J. Smith, CB II, HED
To: L. Propst and M. Fiol, RB, SRRD
Dated: 6/3/92
MRID: No MRID #.

CBRS No.: 10318, DP Barcode D181056
Subject: Bromacil. Protocol for Nature of the Residue in Plants - Citrus Fruit. Dupont Protocol No. AMR 2322-92
From: S. Knizner, CB II, HED
To: M. Fiol, RB, SRRD
Dated: 8/6/92.
MRID: No MRID #.

CBRS No.: 10768; DP Barcode D183799
Subject: Bromacil. Protocol for Nature of the Residue in Citrus Study Guideline 171-4(a). Reregistration Case 0041. Chemical No. 012301
From: S. Knizner, CB II, HED
To: M. Fiol, RB, SRRD
Dated: 2/9/93.
MRID: No MRID #.

MASTER RECORD IDENTIFICATION NUMBERS

Citation for the MRID document referred to in this review is presented below.

42967501 Schneiders, G.E. and Irelan, M.J. (1993) Uptake and Metabolism of [2-^{14}C]bromacil by Orange Trees. Laboratory Project I.D. AMR 2322-92. Unpublished study conducted and submitted by E.I. Du Pont de Nemours and Company (Wilmington, DE). 105 p.

DYNAMAC
CORPORATION
Environmental Services

Final Report

BROMACIL
Shaughnessy No. 012301; Case No. 0041 (CBRS No. 12786; DP Barcode D196552)

TASK 4
Registrant's Response to Residue Chemistry Data Requirements

January 26, 1994

Contract No. 68-D2-0053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
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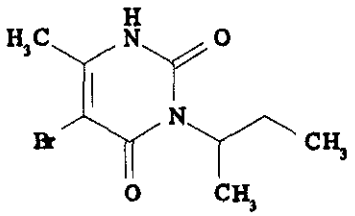
BROMACIL



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Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

E. I. du Pont de Nemours and Company has submitted descriptions of and recovery data for a GLC analytical method with electron-capture detection (GC/ECD) (1993; MRID 42967301) for determining residues of bromacil *per se* in/on oranges and pineapple. The submitted analytical method data are the subject of this evaluation and the Conclusions and Recommendations stated below pertain only to this topic. All other residue chemistry data requirements required in the Second Round Review (SRR) of the Bromacil Reregistration Standard dated 8/15/89 are not addressed herein.

With respect to the residue analytical method requirements for the reregistration of bromacil, the Bromacil Reregistration Standard SRR required that representative samples of pineapple, citrus, and ruminant commodities bearing bromacil residues of concern be subjected to multiresidue protocol V. It was also concluded that because the metabolism of bromacil in plants and animals was not adequately understood, additional validated methods for data collection and tolerance enforcement may be required.

The metabolism of bromacil in citrus is adequately understood. An orange metabolism study (MRID 42967501; DP Barcode D196549) was submitted and it was determined that the residue to be regulated in **citrus** was bromacil. A pineapple metabolism study is in progress and will be available for review by 9/94. The qualitative nature of the residue in ruminants is adequately understood. A poultry metabolism study is presently not required since bromacil is not registered for use on crops that are used as poultry feed.

Tolerances for residues of bromacil in/on plant commodities are expressed in terms of bromacil *per se* [40 CFR §180.210]. No tolerances for animal commodities have been established. Adequate methods are available for tolerance enforcement and data collection. A GLC method with microcoulometric detection is available for tolerance enforcement and is

listed in Pesticide Analytical Method (PAM) Vol. II as Method I. Additional methods deemed adequate for purposes of tolerance enforcement include a GLC method with electron-capture detection, published in PAM Vol. II as Method B, and an improved GLC method using a thermionic nitrogen/phosphorus detector. These methods have not undergone validation by the Agency; therefore, they may be considered only as confirmatory methods for determining residues of bromacil *per se*.

CONCLUSIONS AND RECOMMENDATIONS

1. The modified GLC/ECD method has a more sensitive limit of detection (LOD = 0.01 ppm) than any of the enforcement methods published in PAM Vol. II and is adequate for data collection of residues of bromacil *per se* in/on oranges and pineapple. Acceptable method recoveries were obtained in oranges (83-110%) and pineapple (80-110%) following fortifications with bromacil at 0.01-0.12 ppm.

2. Since bromacil is the residue to be regulated on citrus, CBRS recommends that this modified GLC/ECD method undergo an independent laboratory method validation in accordance with PR Notice 88-5. If the required pineapple metabolism study in progress indicates that additional pineapple metabolites need to be included in the tolerance expression, then adequate residue analytical methods capable of determining these residues must be developed for pineapples.

DETAILED CONSIDERATIONS

Residue Analytical Methods

E. I. du Pont de Nemours and Company submitted descriptions of and recovery data (1993; MRID 42967301) for a GLC analytical method with electron-capture detection (GC/ECD) for determining residues of bromacil *per se* in/on oranges and pineapple. The submission additionally contains details of a confirmatory GLC method with mass selective detection (GLC/MSD) for determining residues of bromacil *per se* in/on similar commodities.

In essence, the submitted method is a modification of a similar GLC/ECD method published in PAM Vol. II as Method B. Briefly, residues of bromacil in/on orange and pineapple samples are extracted with chloroform. After centrifugation, the supernatant is evaporated to dryness and the residue is reconstituted in 0.1 N NaOH. The NaOH extract is then cleaned on a C_{18} SPE column and eluted with 0.1 N NaOH. Residues are partitioned into ethyl acetate and the ethyl acetate extract is analyzed by GC/ECD using a fused silica capillary column. The limit of detection is 0.01 ppm with a limit of quantitation of 0.02 ppm. Sample chromatograms were provided with the submission. This method was validated by fortifying orange and pineapple samples with bromacil at 0.010-0.12 ppm and analyzing the fortified samples as described. The method recovery data are presented in Table 1.

A GC/MSD confirmatory method may be used if bromacil residues are present at or above a level of 0.01 ppm. The detection limit for this confirmatory method is 0.04 ppm with a limit of quantitation of 0.10 ppm. Operating conditions of the GC/MSD method and sample chromatograms were submitted.

Table 1. Recoveries of bromacil in/on oranges and pineapple fortified with bromacil and analyzed by GC/ECD (MRID 42967301).

Commodity	Fortification Level (ppm)	No. of Samples	% Recovery
Oranges	0.01	4	90-110
	0.04	3	95-108
	0.12	3	83-100
Pineapple	0.01	3	80-90
	0.04	3	90-110
	0.12	3	100-108

The modified GLC/ECD method has a more sensitive limit of detection (LOD = 0.01 ppm) than any of the enforcement methods published in PAM Vol. II and is adequate for data collection of residues of bromacil *per se* in/on oranges and pineapple. Acceptable method recoveries were obtained in oranges (83-110%) and pineapple (80-110%) following fortifications with bromacil at 0.01-0.12 ppm.

The qualitative nature of bromacil residues in citrus is understood. Since bromacil is the residue to be regulated on citrus, CBRS recommends that this modified GLC/ECD method undergo an independent laboratory method validation in accordance with PR Notice 88-5. If the required pineapple metabolism study in progress indicates that additional pineapple metabolites need to be included in the tolerance expression, then adequate residue analytical methods capable of determining these residues must be developed for pineapples.

MASTER RECORD IDENTIFICATION NUMBERS

The citation for the MRID document referred to in this review is presented below.

42967301 Amoo, J.S., Walker, D.E., and Irelan, P.J. (1993) Analytical Method for the Quantitation of Bromacil in Citrus Crops (Oranges) and Pineapples by Gas chromatography/Electron-Capture Detection. Laboratory Project I.D. AMR 2465-92. Unpublished study conducted and submitted by E.I. Du Pont de Nemours and Company (Wilmington, DE). 39 p.

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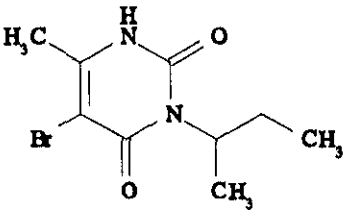

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REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Second Round Review (SRR) of the Bromacil Reregistration Standard dated 8/15/89 required storage stability data to validate the integrity of samples from previously submitted residue field trials used for tolerance assessment. In addition, storage stability data were concurrently required on commodities for which residue field trials are outstanding. In response, E. I. du Pont de Nemours and Company has submitted data (1993; MRID 42967401) depicting the frozen storage stability of residues of bromacil *per se* in/on oranges and pineapple. The submitted storage stability data are the subject of this evaluation and the Conclusions and Recommendations stated below pertain only to this topic. All other residue chemistry data requirements required in the Bromacil Reregistration Standard SRR are not addressed herein.

The metabolism of bromacil in citrus is understood. An orange metabolism study (MRID 42967501; DP Barcode D196549) was submitted and it was determined that the residue to be regulated is bromacil. A pineapple metabolism study is in progress and will be available for review by 9/94. The qualitative nature of the residue in ruminants is adequately understood. A poultry metabolism study is presently not required since bromacil is not registered for use on crops that are used as poultry feed.

Tolerances for residues of bromacil in/on plant commodities are expressed in terms of bromacil *per se* [40 CFR §180.210]. No tolerances for animal commodities have been established. Adequate methods are available for tolerance enforcement and data collection. A GLC method with microcoulometric detection is available for tolerance enforcement and is listed in Pesticide Analytical Method (PAM) Vol. II as Method I. Additional methods deemed adequate for purposes of tolerance enforcement include a GLC method with electron-capture detection, published in PAM Vol. II as Method B, and an improved GLC method using a thermionic nitrogen/phosphorus detector. These methods have not undergone

validation by the Agency; therefore, they may be considered only as confirmatory methods for determining residues of bromacil *per se*. Since bromacil is the residue to be regulated on citrus, CBRS recommends that this modified GLC/ECD method undergo an independent laboratory method validation in accordance with PR Notice 88-5. If the required pineapple metabolism study in progress indicates that additional pineapple metabolites need to be included in the tolerance expression, then adequate residue analytical methods capable of determining these residues must be developed for pineapples.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted storage stability data indicate that fortified residues of bromacil *per se* are stable under frozen storage condition (-15 C) for up to 18 months in/on citrus fruits and pineapple, the only crops on which bromacil is registered for use.

2. A magnitude of the residue study in citrus fruits is presently not required. The registrant did not provide information regarding the duration of storage of citrus samples that were used for tolerance assessment. In consideration of the facts that no bromacil was observed in the citrus metabolism study and that residue samples used for tolerance assessment mostly bore nondetectable (<0.05 ppm) residues of bromacil *per se*, it can be concluded that the citrus fruit field trials are validated by acceptable storage stability data.

3. A pineapple field residue study is ongoing. CBRS recommends that harvested samples from the pineapple field trials be analyzed within 18 months or storage stability data reflecting longer storage intervals will be required.

4. The qualitative nature of the residue in **citrus** is adequately understood. If the pineapple metabolism study in progress indicates that additional metabolites need to be included in the tolerance expression, then additional storage stability data on these residues will be required.

DETAILED CONSIDERATIONS

E. I. du Pont de Nemours and Company has submitted data (1993; MRID 42967401) depicting the frozen storage stability of residues of bromacil *per se* in/on oranges and pineapple. Untreated oranges and pineapple (without tops) that had been obtained commercially were homogenized in liquid nitrogen. After the nitrogen was allowed to evaporate, the samples were stored frozen at -15 C until fortification. Subsamples (25 g) of each commodity were thawed and fortified with bromacil at 0.40 ppm and stored frozen at -15 C. At intervals of 0, 3, 6, 12, and 18 months following fortification, subsamples were collected and analyzed for residues of bromacil *per se*. At each sampling interval, two aged, one freshly fortified, and one untreated control sample of each commodity were analyzed.

Residue analytical methods

The control and fortified samples of oranges and pineapple were analyzed for residues of bromacil *per se* using a GLC analytical method with electron-capture detection (GC/ECD; Enviro-Bio-Tech LTD's SOP EBT208.01; "Standard Operating Procedure for the Determination of Bromacil, Terbacil, and Metabolite Residues in Crops and Soil"). This method is a modification of a similar GLC/ECD method published in PAM Vol. II as Method B. Briefly, residues of bromacil in/on orange and pineapple samples were extracted with chloroform, mixed with water, and the chloroform was removed by rotary evaporation. Residues were taken up in acetonitrile (ACN), partitioned with hexane, and the hexane washes were discarded. The ACN was evaporated to dryness and mixed with 0.1 N NaOH. The residues were then partitioned into ethyl acetate and were determined by GLC/ECD using a SPB-608 fused silica capillary column. The limit of quantitation of the method for each commodity was 0.05 ppm; the limit of detection presumably (not specified) was 0.04 ppm.

The results of storage stability study are presented in Table 1. Apparent residues of bromacil were <0.04 ppm in/on 10 untreated samples of oranges and pineapple.

Table 1. Recoveries of bromacil in/on samples of oranges and pineapple fortified at 0.40 ppm and stored frozen at -15 C.

Storage Interval (Months)	Percent Recovery [a]	Concurrent Method Recovery (%)
Oranges		
0	74, 83	55, 94
3	40-97 (3 samples) [b]	84
6	115, 124	115
12	105, 109	117
18	89, 92	97
Pineapple		
0	84, 98	64, 106
3	59-107 (3 samples) [b]	40, 115
6	95, 120	87
12	64, 104	113
18	92, 95	100

[a] Recoveries are not corrected for control values.
[b] Includes recovery values from duplicate analysis.

The submitted storage stability data indicate that fortified residues of bromacil *per se* are stable under frozen storage condition (-15 C) for up to 18 months in/on citrus fruits and pineapples, the only crops on which bromacil is registered for use.

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A pineapple field residue study is ongoing. CBRS recommends that harvested samples from the pineapple field trials be analyzed within 18 months or storage stability data reflecting longer storage intervals will be required.

The qualitative nature of the residue in **citrus** is adequately understood. If the pineapple metabolism study in progress indicates that additional metabolites need to be included in the tolerance expression, then additional storage stability data on these residues will be required.

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42967401 Schneiders, G.E. and Irelan, M.J. (1993) Stability of Bromacil in Stored Analytical Samples. Laboratory Project I.D. AMR 2282-92. Unpublished study conducted and submitted by E.I. Du Pont de Nemours and Company (Wilmington, DE). 53 p.

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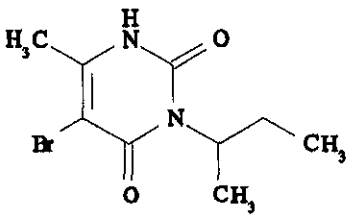

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